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Abstract: Bacterial anchoring to limestone rocks is thought to occur by selective adsorption of biomolecules found in the extracellular matrix, such as polysaccharides. Here we study the adsorbed structure of a model matrix polysaccharide, sodium alginate, at the calcite/water interface using neutron reflection (NR). Sodium alginate was found to form highly hydrated layers extending up to 350 Å into solution at concentrations up to 2.5 ppm (the inflection point of the adsorption isotherm). The adsorption of alginate was driven by dissolution of the calcite surface through complexation of free calcium ions. This was shown using two alginates with differing ratios of sugar residues. Alginates with a higher proportion of guluronic acid (G) have a higher affinity for calcium ions and were found to cause the surface to dissolve to a greater extent and to adsorb more at the surface when compared to alginates with a higher proportion of mannuronic acid (M). Adding magnesium to the high G alginate solution reduced dissolution of the surface and the adsorbed amount. In this work, we have shown that polysaccharide adsorption to sparingly soluble calcite interfaces is closely related to polymer conformation and affinity for free calcium ions in solution.



*Stuart Clarke,
Fellow of Jesus College,
Reader,
BP Institute,
Department of Chemistry*

Dear Professor Berti,

Please find enclosed our manuscript on the adsorption of sodium alginate to calcite surfaces as studied by neutron reflection.

We believe this is the first report of biomolecule adsorption to calcite using this technique and as a result we are able to characterise the amount and structure of the adsorbed layer. In particular, the role of calcium binding and alginate composition is highlighted.

The Journal of Colloid and Interface Science has a long history of publishing neutron reflectivity of soft matter studies and has previously supported our earlier work on neutron reflectivity of calcite interfaces. We feel that this study will be of interest to your readers, particularly in related areas, such as biomineralisation.

This research has not been published elsewhere and does not contain any work being considered for other journals.

Please find below a list of possible reviewers.

Name	Affiliation	Email
Professor Fiona Meldrum	University of Leeds, UK	f.meldrum@leeds.ac.uk
Professor Susan Stipp	Danmarks Tekniske Universitet, Denmark	stipp@fysik.dtu.dk
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Professor Neal Skipper	University College London, UK	n.skipper@ucl.ac.uk
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If you require any further information please do not hesitate to contact me.

Yours Faithfully,

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DEPARTMENT OF PHARMACY



1 JULY 2020

Dear Professor Berti,

Thank you for your evaluation of our manuscript "The effect of alginate composition on adsorption to calcium carbonate surfaces" Ref: JCIS-20-3387.

We have addressed the comments raised by the editorial office and the reviewers below and made highlighted changes in the updated manuscript.

Reviewers' comments:

Reviewer #1: The authors report on the adsorption of sodium alginate on a calcite surface in water.

They used a contrast series of neutron reflectometry measurements to compare adsorption of two alginate samples with low and high content of guluronic acid, concluding that alginates with a higher guluronic acid content adsorbed more.

I found this manuscript really well written, clear and convincing. The reflectometry measurements, which are central to the paper, were very well described. I would definitely recommend accepting this manuscript for publication, with minor revisions.

We thank the reviewer for his/her positive appraisal of our manuscript.

I noted the following recommendations. Those would help the reader understand the interpretation of the data a little better.

1- Page 10, Line 2: "Errors were calculated using the bootstrap error analysis [...]"

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MOB 45 42 49 48 25

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Would it be possible to add a couple of lines describing what this method is?

Within the RasCAL program the Monte Carlo error is calculated by randomising the parameters within the set limits and calculating the best fit to the data, this is performed 100 times and the error is calculated from a histogram of obtained values.

The manuscript has been updated to read:

“Errors were calculated using the bootstrap error analysis in the RasCAL program, where random parameter values are generated and a fit performed 100 times to generate confidence intervals. The parameter errors were then used to calculate the error in adsorbed amount, assuming the maximum and minimum possible values.” Page 9 Line 21 – Page 10 Line 2

2- Page 10, Line 4: "The SLDs [...] were ALL fixed according to the literature values (Table 1)." (Emphasis mine)

Table 1 is not part of the main manuscript. Table S1 is probably meant.

Minor clarity issue: This sentence is also confusing because it is followed by a statement that contradicts it. For clarity, it might be best to point out the literature SLDs in Table S1 and mention that these values were used whenever possible. Then the description of how the H₂O SLD was determined can follow.

We thank the reviewer for noticing the mistake. This has been updated in the manuscript.

The manuscript has been updated to:

“The SLDs of substrate, subphase, and adsorbate were fixed, where possible, according to the literature values (Table S1).” Page 10 Lines 2-3

We have clarified that H₂O contrast is a fitting parameter in the manuscript text as below:

“The two (D₂O and H₂O) data sets were fitted simultaneously using only two fitting variables, surface roughness and H₂O contrast.” Page 11 21-22

A footnote has also been added to Table S1 in the supporting information explaining that it was sometimes necessary to fit the H₂O contrast. SI Page 2

3- Page 11, Line 4: "[...] this difference is close to the error [...]"

This is not really true. Looking at Fig 2, the difference is definitely statistically significant. I don't think there's a problem with that. It wasn't clear why this needed to be brought up. I would suggest dropping the statement about the difference being close to the error.

We agree with the reviewer that the difference is significant, we have removed this sentence from the manuscript. Page 10 Line 16.

4- Page 14, Line 2. *"The SLD of the subphase in the H₂O was higher than the literature [...]"*

How was that SLD value determined? I notice that this value doesn't have an uncertainty, unlike most other model parameters.

The SLD value for H₂O was determined by fitting the contrast as a parameter in a similar way to the other tabulated parameters such as surface roughness. Due to the lack of total reflection for contrasts that are lower than the substrate (Calcite = $4.69 \times 10^{-6} \text{ \AA}^{-2}$) it is not possible to fix the contrast without fitting, however, we have found that the fit to the contrast is very robust as the intensity of reflection at low Q is not affected by the adsorbed layer at the surface. We obtain an error of $0.05 \times 10^{-6} \text{ \AA}^{-2}$ for MVM and $0.07 \times 10^{-6} \text{ \AA}^{-2}$ for MVG using the bootstrap error analysis described above and have added this value to the manuscript as below:

"The SLD of the subphase in the H₂O was fitted to be higher ($2.28 \pm 0.05 \times 10^{-6} \text{ \AA}^{-2}$) than the literature value of $-0.56 \times 10^{-6} \text{ \AA}^{-2}$, due to incomplete exchange, as discussed above." Page 13 Line 15 – Page 14 Line 1

We have also stated the fitted H₂O contrast for the MVG alginate with error

"The SLD of the H₂O contrast was again found to be higher than literature ($3.28 \pm 0.07 \times 10^{-6} \text{ \AA}^{-2}$) as for MVM alginate." Page 14 Lines 7-8

5- Description of the fitted results

The reflectometry modeling is well described. Figure 5 is very good. To be able to better judge the fits, it would be nice to see the fitted SLD distributions. This way the reader can visually assess how the roughness and adsorbed layer were modeled.

We thank the reviewer for his/her positive comment. In the manuscript we avoided using SLD profiles as they are often only useful to experts in the field, hence we chose instead to show volume fractions. We agree that it is useful to equate the fits to the calculated scattering length density profiles and have included them as new figures in the supporting information (Figure S3). SI Page 4

Sentences have been added to the manuscript to point to this:
 “Corresponding SLD profiles are provided in the supporting information (Figure S3).” Page 12 Line 15

Reviewer #2: The authors present interesting fundamental studies on the formation of alginate coatings onto calcium carbonate surfaces. Neutron reflection studies have been applied to study the roughness, the thickness and the amount of adsorbed alginates. These data should be useful to researchers working with alginate based coatings, but I provide a number of comments which should improve the quality of this work further.

We thank the reviewer for his/her positive appraisal of our manuscript.

Page 4: in the last paragraph, the authors discuss bacteria and their role in production of alginates. This paragraph appears not to be connected with previous or next paragraphs.

Having reread the introduction with this in mind we can see that the flow is not ideal. In order to better link the red thread through the introduction we have moved this paragraph above the previous one. The flow now discusses the bacteria as a potential source of alginate, describes the bacterial alginates and then talks more about the physics behind why the different alginates have different properties. Page 4 Lines 4-10

This is a nice fundamental study, but I wonder why the authors have not used traditionally applied infrared spectroscopy to confirm the presence of materials. Also, often the adsorption studies are done with atomic force microscopy (AFM) visualization of the surface. It would be helpful to understand the complete picture, i.e. the roughness of the surface. It would have helped to confirm the presence of calcite and it would have ideally fit into conclusions drawn from Figure 5 (C).

If AFM is not available, perhaps some electron microscopy scans can be added, again, to visualize the surface.

We have chosen in this study to focus on the use of neutron reflectivity as it provides unparalleled information on the buried surface structure parallel to the interface, through this we are able to confirm the presence of alginate at the surface and determine a surface roughness over the whole crystal surface. We tested the hypothesis of pure surface roughening and pure adsorption but the only way to fit to the data adequately was to assume both surface roughening and adsorption. Hence we are reasonably convinced on the presence of the materials.

We are currently attempting to develop surface IR of calcite water interfaces by combining SFG/RAIRS, however, this is non-trivial due to the birefringence and peak ratio being affected by crystallographic axis orientation. We hope to include this in future work but do not feel that it would add significant findings to the current study.

Perry *et al.* studied the AFM surface in contact with alginic acid in a stand-alone study (Perry IV, T. D.; Duckworth, O. W.; McNamara, C. J.; Martin, S. T.; Mitchell, R. Effects of the Biologically Produced Polymer Alginic Acid on Macroscopic and Microscopic Calcite Dissolution Rates. *Environ. Sci. Technol.* **2004**, 38 (11), 3040–3046.

<https://doi.org/10.1021/es035299a>). Although they only used one type of alginic acid, they were able to study the etch pit formation and found that the obtuse steps of the retreat rate increased dramatically upon interaction with alginate (Page 20 Lines 13-18). Although it would be nice to include information on the effects of MVM and MVG to the lateral surface roughening we feel that this would require a large amount of method development and provide only an incremental increase in knowledge of our system.

In description of adsorption isotherms, please clarify what handling steps have been performed with calcite itself.

The calcite powder was used as received, weighed into a 500 mL polypropylene container and homogenised. The slurry was then measured into centrifuge tubes by weight.

The sentence describing the calcite preparation in the manuscript has been clarified to:

“The slurry was then weighed into centrifuge tubes and increasing amounts of alginate stock solution was added” Page 6 Lines 19-20

On page 19, the authors state that polyelectrolyte adsorption to oppositely charged surfaces is a complicated process, citing papers from 90-ies. Since

then, this process is much better understood. Subsequently, the structure of polyelectrolytes is discussed. This paragraphs can be certainly improved, but bringing the following: internal structure of polyelectrolytes: PhysChemChemPhys 2006, 8, 5012-5033; ions contents in polyelectrolyte: Maromolecul. 2019, 52, 9149-9159; a better stability of Ca-crosslinked alginates for biological application: J. Mater. Chem. B 2019, 7, 6778-6788.

We have improved the paragraph to include extra elements of polyelectrolyte adsorption as the reviewer recommends. The following has been added:

Entropic effects are also expected to play a major role in the adsorption of oppositely charged polyelectrolyte adsorption through the release of surface bound charge compensating counterions.⁴⁰ Page 20 Lines 3-5

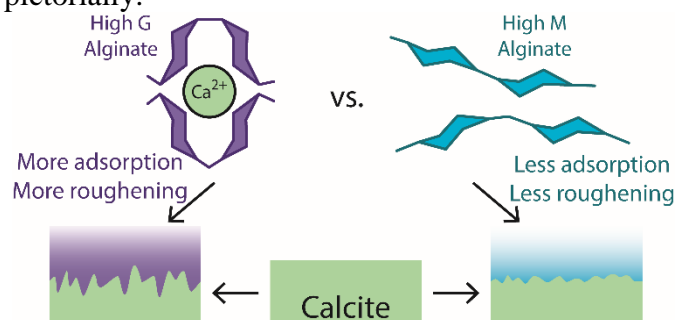
This is a nice study, but it can be better linked with the actual potential application. Dissolving calcium carbonate surfaces is relevant, but the formation of the polysaccharide coatings crosslinked with multiple di-valent atoms (like Ca, Mg) have been shown to be used for biomedicine as injectable materials: Biomed. Mater. 2017, 12, 025015 and Int. J. Biolog.Macromol. 2018, 118, 1257-1266.

We thank the reviewer for highlighting a potential application and a wider audience for this paper. We have included the references in the discussion as follows:

“These results can be used to understand the formation of highly charged polysaccharide coatings and hydrogels in the presence of divalent cations for biodegradable tissue scaffolds or injectable biomaterials.⁴⁵⁻⁴⁷” Page 21 Lines 6-8

Other changes:

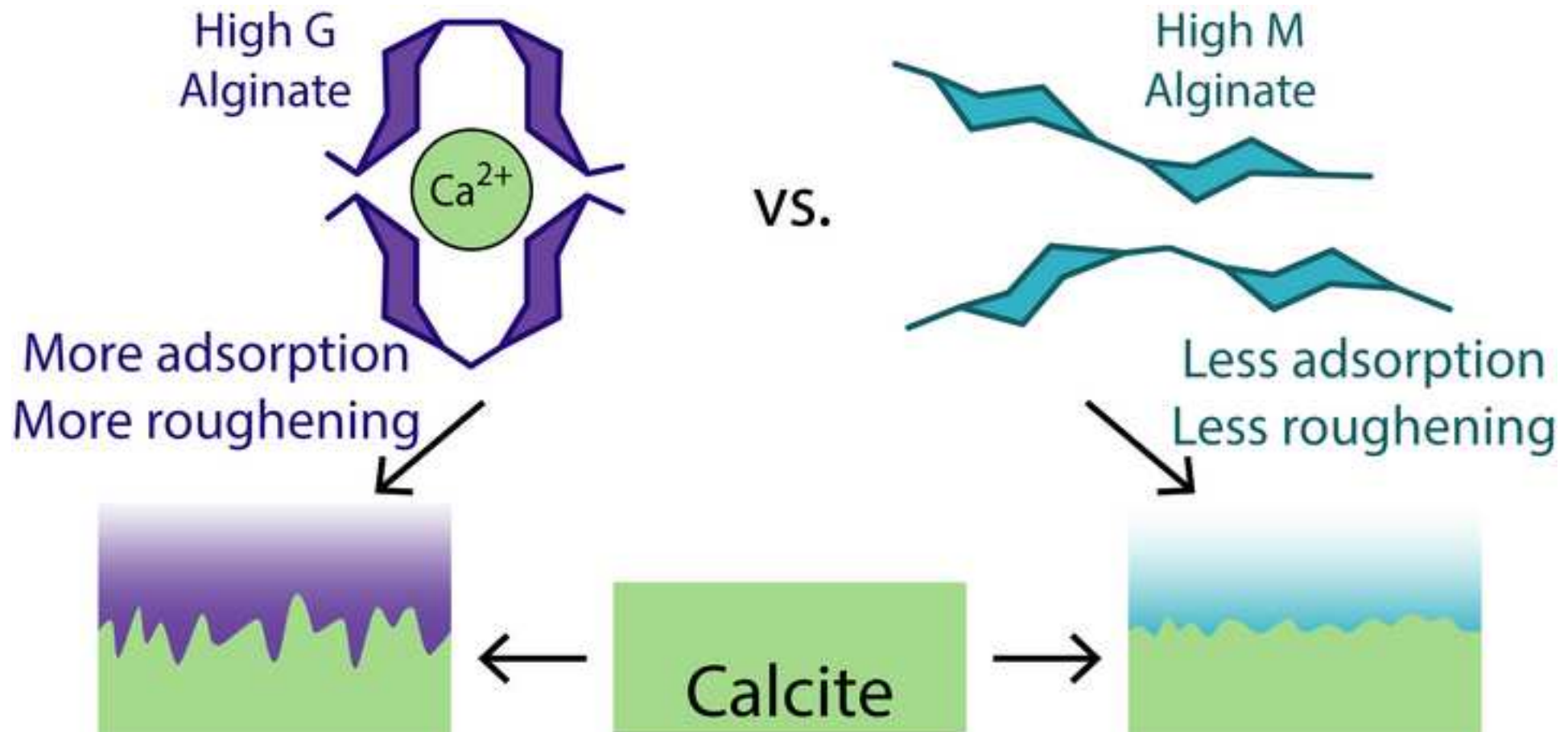
We have updated the graphical abstract to better describe the outcome of the experiment pictorially:



With these changes and revisions, we hope that the paper is now be acceptable for publication in Journal of Colloid and Interface Science.

Yours sincerely,

Kathryn Browning
Assistant Professor



The effect of alginate composition on adsorption to calcium carbonate surfaces

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ABSTRACT

Bacterial anchoring to limestone rocks is thought to occur by selective adsorption of biomolecules found in the extracellular matrix, such as polysaccharides. Here we study the adsorbed structure of a model matrix polysaccharide, sodium alginate, at the calcite/water interface using neutron reflection (NR). Sodium alginate was found to form highly hydrated layers extending up to 350 Å into solution at concentrations up to 2.5 ppm (the inflection point of the adsorption isotherm). The adsorption of alginate was driven by dissolution of the calcite surface through complexation of free calcium ions. This was shown using two alginates with differing ratios of sugar residues. Alginates with a higher proportion of guluronic acid (G) have a higher affinity for calcium ions and were found to cause the surface to dissolve to a greater extent and to adsorb more at the surface when compared to alginates with a higher proportion of

mannuronic acid (M). Adding magnesium to the high G alginate solution reduced dissolution of the surface and the adsorbed amount. In this work, we have shown that polysaccharide adsorption to sparingly soluble calcite interfaces is closely related to polymer conformation and affinity for free calcium ions in solution.

KEYWORDS

- Calcium Carbonate
- Calcite
- Biomineralization
- Alginate
- Polysaccharides
- Mannuronic Acid
- Guluronic Acid
- Adsorption
- Neutron Reflection
- Interfaces

INTRODUCTION

Most of the Earth's calcite (CaCO_3) is of biogenic origin from carbonaceous marine organism sedimentation. Calcium carbonate biomineralisation provides structure throughout Nature from avian eggshells to marine molluscs and coccoliths.^{1,2} The fine control of crystal morphology, phase and grain size is moderated through the production of various biomolecules, which also affect the mechanical properties.^{2,3} The precise mechanism by which these molecules interact with the crystallizing medium through nucleation and growth is an area of current interest in several areas, including amorphous calcium carbonate precursor phases (ACC).³⁻⁹

Calcium carbonate can be precipitated in several different polymorphs of varying stability. The most stable polymorph under ambient conditions is calcite; it can form large defect-free crystals, making it suitable as the focus of this experimental study. It is sparingly soluble in water with a solubility product at room temperature of $K_{\text{sp}} = 3.7 \times 10^{-9} \text{ mol dm}^{-3}$.¹⁰ This dissolution complicates the analysis of adsorption to calcite surfaces as acidic conditions can lead to a highly

dynamic interface. The dissolution or precipitation of calcite is closely linked with other aqueous equilibria that determine the extent of dissolution of the surface.¹¹

The driving force for adsorption of polyanionic polysaccharides is expected to be the affinity of the charged carboxylate groups for calcium on the positively charged calcite surface. Calcium cations released from the calcite surface are sequestered to the sugar forming a complex. This process in turn removes calcium from the solution shifting the equilibrium such that more calcium carbonate solid is dissolved. Polyanionic polysaccharides have been shown to affect the crystal growth structure of calcium carbonate by interacting with the mineral surface suggesting specific interactions with the calcite surface.¹² Furthermore, negatively charged sugars secreted by bacteria into the extracellular matrix may aid anchoring of the cell to calcitic limestone rocks in marine environments.^{13,14}

Sodium alginate has been chosen for this study as a model polysaccharide due to its well-understood structural characteristics and calcium binding properties, allowing the interplay between complex-mediated surface dissolution and polymer adsorption to be investigated.¹⁵

Alginates are a major structural component of marine algae and secreted by some bacteria, most notably *Pseudomonas aeruginosa* and *Azotobacter vinelandii*.¹⁶ As schematically illustrated in Figure 1, sodium alginate is a copolymer of two epimers, mannuronic acid (M) and guluronic acid (G), which differ only by the conformation of the 1,4 glycosidic link. The two sugar residues can be found either as homopolymeric blocks of each sugar or as alternating MG sequences.¹⁷ The primary structure of alginate affects the macromolecular properties greatly. GG blocks are rigid as the axial linkage decreases movement and provides a binding site for divalent cations. Conversely, MM blocks are joined via an equatorial glycosidic linkage are therefore

relatively free.¹⁷ The molecular conformation of the pairs, MM, MG and GG are included in Figure 1.

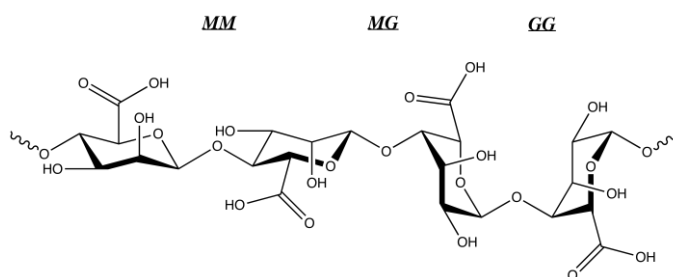


Figure 1: Molecular structure of the alginic acid showing the various links between the two epimers, MM, MG and GG diblocks.

Two gram negative, alginate producing, bacteria, *Pseudomonas aeruginosa* and *Azotobacter vinelandii*, secrete alginates with different compositions, macromolecular properties and functions. *P. aeruginosa* secretes alginate as a protective envelope and adhesive molecule in biofilm formation in the lung, these alginates have little to no G blocks.¹⁶ Conversely, *A. vinelandii*, a soil bacterium that forms exopolysaccharide cysts in soil to protect from mechanical stress and desiccation during dormancy, produces alginates with high ratios of GG blocks and alternating sequences in the outer protective layer.¹⁶

The more rigid GG sections provide well-defined chelation sites, which leads to gelation on addition of calcium. The more flexible MM and MG blocks deform to allow adjacent GG disaccharide units to line up form a chelating cage composed of four G saccharide units.¹⁸ This ability to co-ordinate is strongly linked to conformational structure, alginates with a higher proportion of G (and G blocks) gel at lower concentrations of calcium ions and form stronger and more brittle gels.^{19,20} Grant *et al.* defined this guluronate-Ca interaction the ‘egg-box model’ due to the alignment of guluronate chains along a plane of calcium ions.^{17,21} In contrast to

1 calcium, the binding of magnesium with alginate molecules is reported to be a non-specific
2 interaction.^{22,23} Magnesium alginate is soluble and does not strongly interact with any specific
3 binding sites.^{23,24}

4 In this study we have used neutron reflection and bulk solution depletion isotherms to compare
5 the adsorption of two different sodium alginate samples with either a low (MVM) or high
6 (MVG) content of guluronate residues to calcium carbonate surfaces. Considering the higher
7 affinity of guluronate residues to calcium ions in solution, the two alginates are expected to show
8 very different surface adsorption profiles in terms of both adsorbed amount and structure.
9 Neutron reflectivity allows the measurement of complex adsorbed structures at the solid/liquid
10 interface, something which is challenging using other techniques. The study of the solid/liquid
11 interface using neutron reflection has been reasonably limited to a small number of single crystal
12 interfaces, mainly silicon, quartz and sapphire.²⁵ Recently the number of mineral surfaces used in
13 reflection experiments has widened to include calcite, however, previous work has focused on
14 the adsorption of industrial chemicals and surfactants at the calcite/liquid interface.^{26–28} Here we
15 aim to investigate the adsorption of biopolymers to calcite surfaces in aqueous environments to
16 show how polymer conformation and calcium ion dissolution from the calcite surface can affect
17 adsorbed amount and structure at the interface.

18 EXPERIMENTAL

19 MATERIALS

20 All experiments were carried out using two ultrapure and well-characterised alginates (Pronova
21 UP Test kit) obtained from Novamatrix. The two alginates tested were of medium viscosity
22 (>200 mPa·s) and high molecular weight (>200 kDa) and differ by the ratio of the two sugar

1 epimers. The high guluronate sample (Medium Viscosity Guluronate, MVG) was reported to
2 have a viscosity of 263 mPa·s and contain 69 % guluronate residues. The high mannuronate
3 sample (Medium Viscosity Mannuronate, MVM) was found to have a higher viscosity (553
4 mPa·s) with a mannuronic acid content of 54 %. All values quoted here were obtained from the
5 manufacturers certificate of analysis of the batch used.

6 Calcium carbonate powder ($\geq 99\%$) used for adsorption isotherms and to saturate water for NR
7 experiments was purchased from Sigma Aldrich. Calcite crystals used in neutron reflection
8 experiments were optically clear Iceland Spar crystals obtained from P&E Export Ltd, Brazil.
9 The large rhombohedral crystals were cut to a cuboid shape (40 x 55 x 15 mm) and polished to a
10 low roughness by Crystran, Poole, UK. The surface for reflectivity was the most stable $\{10\bar{1}4\}$
11 face, the crystallographic plane found at the surface of the natural rhomb. Attempts were made to
12 cleave the surface rather than polish, however, it was not possible to obtain an area large enough
13 for reflectivity studies (roughly 20 cm²) without introducing large steps, causing the cells to leak.
14 The roughness of the polished crystals was assessed by X-ray reflectivity on a Rigaku SmartLab
15 X-ray diffractometer (ISIS, Rutherford Appleton Laboratory, Didcot, UK). This measurement
16 gave an approximate indication of the quality of the surface (data shown in supporting
17 information, Figure S1). Crystals, which had an X-ray roughness less than 15 Å, were then used
18 in neutron reflectivity experiments.

19 ADSORPTION ISOTHERMS

20 Isotherm measurements were carried out using a solution depletion batch method. 2g of calcium
21 carbonate powder was homogenised with 498g ultrapure water using a Silverson L4R shear
22 mixer for 10 minutes. The slurry was then weighed into centrifuge tubes and increasing amounts

of alginate stock solution was added. The samples were agitated overnight and centrifuged at 15,000 G for 20 minutes. The supernatant was removed and filtered through a 0.45 µm polyethersulfone syringe filter (QMX, UK). The Total Organic Carbon content of the supernatant was measured using a GE InnovOx TOC Analyser. During analysis, the supernatant was acidified and purged with nitrogen to remove traces of inorganic carbonates. The concentration of non-purgeable carbon remaining in the supernatant was then used to calculate the adsorbed amount of alginate.

NEUTRON REFLECTIVITY

The experiment is performed by illuminating a large flat crystal with a collimated beam of neutrons, which reflect and/or refract at the solid/liquid interface. The intensity of the reflected beam is then measured as a function of wavelength and reflected angle. In this experiment, the D17 reflectometer at the Institut Laue-Langevin, France,²⁹ was used in the time-of flight (TOF) mode where a polychromatic beam of neutrons is directed at the sample and the wavelength determined by the time taken for neutrons to reach the detector from the source. The determined wavelength values are converted to a momentum transfer vector, Q , calculated from equation 1.

$$Q = \frac{4\pi \sin\theta}{\lambda} \quad 1$$

The use of the TOF method on D17 allows the entire scattering range of interest of the reflectivity curve to be measured using only two incident angles, 0.6° and 2.0°, to cover the Q range of interest (0.006-0.2 Å⁻¹). To ensure no contribution from the edges of the sample, a constant beam footprint of 30 x 45 mm² was maintained on the sample surface. The dQ/Q resolution varied between 1.5-10% over the Q range measured, during data fitting a value of 4% resolution was used. The data reduction was performed using the COSMOS software.³⁰

The calcite crystals were cleaned by UV-Ozone treatment (BioForce Nano Procleaner). After irradiation, a small drop of filtered calcium carbonate saturated water was placed on the surface to assess wettability. If the droplet spread the crystal was considered clean, placed immediately against a Teflon trough and sealed in a neutron reflectivity cell (details of cell reflection sample cell are given in Stocker *et. al.*²⁶). Sample changes were carried out by draining the sample cell by pipette (3 mL cell volume) and refilling in-situ with the required solution, this process was repeated three times and the cell sealed with Teflon plugs.

ANALYSIS OF REFLECTIVITY DATA

The shape of the reflectivity curve at a single interface is determined by the surface roughness and the change in neutron refractive index upon moving from one medium to the next through the surface. Neutron reflection theory is outlined elsewhere.³¹ A particularly powerful aspect of neutron reflection is the seemingly random nature of neutron scattering lengths across the periodic table and between isotopes. The scattering length density (SLD) of a material is calculated using equation 2 where N is the number density of atoms in a material and b_i is the scattering length of each nucleus.

$$\rho = \sum N b_i \quad 2$$

The isotope effect is most clearly seen by the very different SLDs of H₂O and D₂O, $-0.56 \times 10^{-6} \text{ \AA}^{-2}$ and $6.35 \times 10^{-6} \text{ \AA}^{-2}$, respectively. Using isotopic substitution it is possible to study different reflectivity profiles in the same chemical environment. In this work, we have measured reflectivity profiles of the calcite/alginate solution in H₂O, D₂O, and a mixture of the two to form a solution that has the same scattering length density of the calcite crystal ($4.69 \times 10^{-6} \text{ \AA}^{-2}$). This combination of solution contrasts is important to constrain the structural model. By using a

1 solution with the same SLD as the calcite, a reflected signal is only seen from the adsorbed
2 alginate (alginate SLD= $2.6 \times 10^{-6} \text{ \AA}^{-2}$, calculated from the expected molecular formula and
3 specific gravity).

4 The measured reflectivity profile is then analysed by comparison of the experimental data with a
5 model structure. The general approach is to constrain the structural model as much as possible to
6 fit the data. If a single layer is found not be sufficient to fit the experimental data, adsorbed
7 layers can be broken into several smaller blocks/layers to describe adsorbed structures in more
8 detail. In some cases a mathematical description of the SLD profile is appropriate, such as
9 exponential or Gaussian.³² In all of these cases, the total reflected intensity from adsorbed layers
10 is calculated using the matrix method developed by Abeles and used in reflectivity fitting
11 programs such as RasCAL.³³

12 The polymer segment density distribution normal to the surface was modelled in several ways,
13 such as discrete layers, exponential decrease from the surface, or a Gaussian distribution. The
14 best fit to the data in all cases was found using the Gaussian distribution model. The SLD profile
15 through the interface was modelled by dividing a 300 Å region into 10 Å sections with an
16 interlayer roughness fixed to 5 Å to smooth out each interface. The volume fraction of alginate
17 was then calculated in each section and converted to an SLD by assuming the rest of the layer
18 was filled with the subphase water. In all adsorbed alginate datasets it was observed that the
19 volume fraction of alginate had reached background well before the model cut off at 300 Å
20 except for the 2.5 ppm MVG where the model had to be extended to 350 Å to reach background.
21 Using the modelled volume fraction distribution, an adsorbed amount was obtained from the area
22 under the Gaussian polymer segment density distribution curve. Although it was possible to fit a
23 small variation in the Gaussian profile, in all cases the distribution was found to conserve the

area under the curve, within error, providing a good test of the robustness of the values for adsorbed amount. Errors were calculated using the bootstrap error analysis in the RasCAL program, where random parameter values are generated and a fit performed 100 times to generate confidence intervals. The parameter errors were then used to calculate the error in adsorbed amount, assuming the maximum and minimum possible values. The SLDs of substrate, subphase, and adsorbate were fixed, where possible, according to the literature values (Table S1). During the experiment it was noted that there was incomplete exchange of the liquids within the cells as the SLD of the H₂O subphase could not be fitted to the literature value, but rather a mixture of H₂O containing some D₂O, only when the liquid in the cell was exchanged from D₂O to H₂O in situ (despite repeated exchanges). This may be due to insufficient mixing of the two liquids due to the density difference in the cell and the high contact angle between the water and the hydrophobic surface of the Teflon trough.

RESULTS

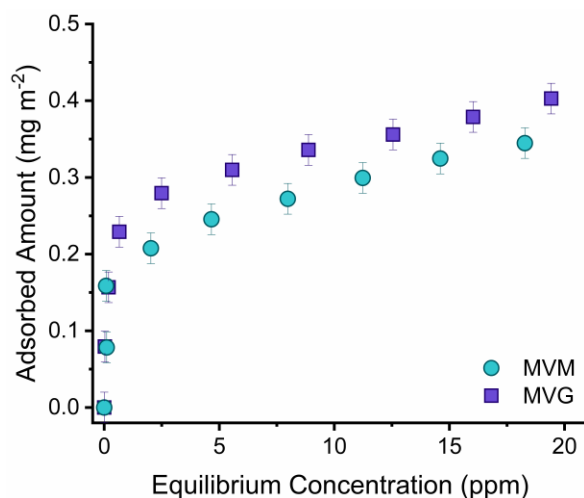


Figure 2: Isotherm data of sodium alginate adsorption to calcite powder in water as measured by total organic carbon analysis (TOC). MVM alginate is shown as green circles and MVG as purple squares.

Figure 2 presents bulk solution depletion isotherms for the adsorption of MVM (green circles) and MVG (purple squares) to calcium carbonate powder. It can be seen that there is a small difference between the two alginate solutions with MVG adsorbing more to the surface than MVM. At low concentrations, all alginate is adsorbed as seen from a steep initial increase in adsorbed amount before the turning point of the isotherm at an equilibrium concentrations of roughly 2.5 ppm. At higher concentrations, the slope becomes shallower but not flat suggesting that the adsorption does not form a monolayer at the surface. There may be some precipitation of the alginate at higher concentrations as the calcium is dissolved into solution forming insoluble calcium alginate. The interaction between the calcium carbonate surface and alginate is observed to be weak due to the low adsorbed amount at equilibrium. This effect could be accentuated by the sample preparation for TOC analysis. In order to remove calcium carbonate powder from the supernatant, the samples are centrifuged at 15,000 G for 20 minutes which is likely to shear off any loosely bound alginate from the surface leaving only the tightly bound molecules that are effectively irreversibly adsorbed by attachment at multiple points.

NEUTRON REFLECTION

Each calcite crystal was first measured in three saturated calcium carbonate water contrasts, D₂O, H₂O, and water contrast matched to calcite ($\text{CMC} = 4.69 \times 10^{-6} \text{Å}^{-2}$). A representative dataset is shown in the supporting information (Figure S2). Previous experiments have shown that the surface roughness of calcite does not increase over time in the presence of water saturated with calcium carbonate powder.²⁷ The surface cleanliness was confirmed by the absence of any reflected signal above the background level in the CMC contrast (not shown). Measuring the reflectivity in contrast matched water is especially useful due to the high sensitivity to adsorbed layers at the surface. Unlike silicon crystals, which always have a surface

oxide layer and always show a signal in contrast matched water, if the surface of calcite is completely clean there will be no signal recorded. The two (D_2O and H_2O) data sets were fitted simultaneously using only two fitting variables, surface roughness and H_2O contrast. All bare calcite crystals for the study were successfully fitted to a single interface model and the surface roughness varied from $8.5 \pm 0.6 \text{ \AA}$ to $9.4 \pm 0.3 \text{ \AA}$.

Before adsorption measurements using neutron reflection it is important to completely characterise the surface that the adsorbate will bind to. Unlike many other surfaces used in reflection the calcite surface can be dynamic and of biogenic origin. Measurements of the bare surface are therefore useful to assess the cleanliness of the substrate and provide an initial roughness characterisation. When calcite is cleaved or polished, high-energy bonding sites are exposed. In air, these sites are likely to be contaminated with species from the atmosphere, however, this bonding of contaminants is generally weak and upon immersion, water molecules compete, removing the contaminants and hydrating the surface with pH sensitive groups.^{11,26,34}

ALGINATE ADSORPTION

Figure 3 presents experimental neutron reflectivity data of the calcite/water interface in the presence of (a) 2.5 ppm MVM and (b) 2.5 ppm MVG alginate in three contrasts, D_2O (purple squares), H_2O (green circles), and water contrast matched to calcite (CMC, yellow triangles). Corresponding SLD profiles are provided in the supporting information (Figure S3). Alginate solutions were introduced to the sample cells at concentrations of 0.5 and 1 ppm in D_2O . Three water contrasts were recorded for the highest concentration, 2.5 ppm. The concentration of 2.5 ppm was chosen to be just above the turning point of the plateau for both MVM and MVG

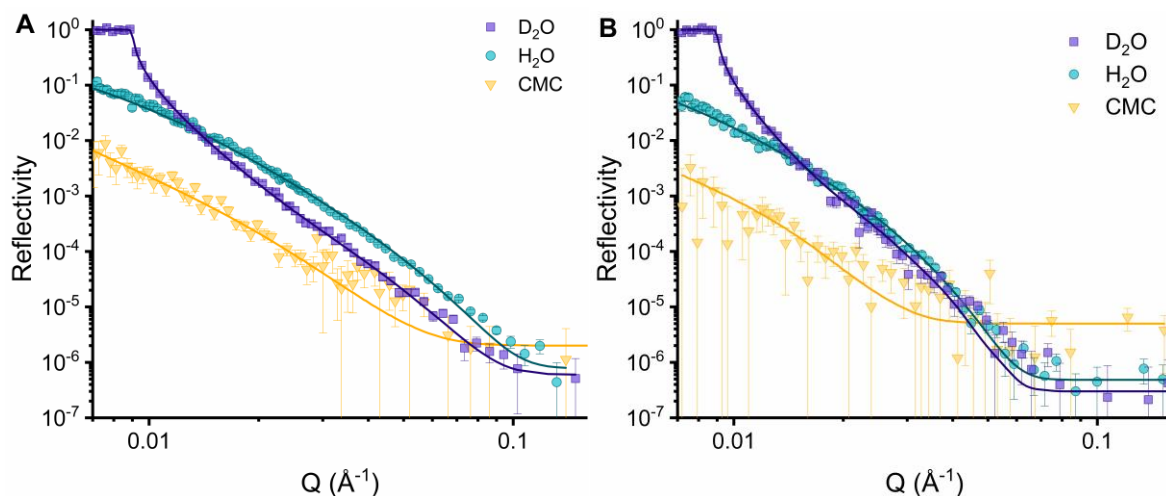


Figure 3: Experimental neutron reflectivity data of the calcite/water interface in the presence of **A** 2.5 ppm MVM and **B** 2.5 ppm MVG alginate in three contrasts, D₂O (purple squares), H₂O (green circles) and water contrast matched to calcite (CMC, yellow triangles). Calculated fits to the data are overlaid as solid lines.

Upon introduction of alginate the intensity of the reflectivity in both the H₂O and D₂O contrasts drop, suggesting that the surface is roughening and/or a diffuse layer is adsorbed. A signal is also seen in the contrast matched water indicating an adsorbed layer was present at the surface. This signal in the contrast matched water is purely from any adsorbed material at the interface and therefore has a rather low signal-to-noise ratio and large error bars. The 2.5 ppm MVM data was fitted to a Gaussian layer model with an adsorbed amount of $1.6 \pm 0.4 \text{ mg m}^2$ and a surface roughness of $16.1 \pm 0.9 \text{ \AA}$. The volume fraction of alginate at the surface was found to be 0.098 showing that the adsorbed layer is highly hydrated at the interface. The volume fraction decreases away from the surface into solution before reaching zero between 200 and 250 \AA . As

the concentration of MVM alginate increases, the amount adsorbed at the surface initially increases and begins to level off reaching $1.6 \pm 0.4 \text{ mg m}^{-2}$ at 2.5 ppm MVM. The SLD of the subphase in the H_2O was fitted to be higher ($2.28 \pm 0.05 \times 10^{-6} \text{ \AA}^{-2}$) than the literature value of $0.56 \times 10^{-6} \text{ \AA}^{-2}$, due to incomplete exchange, as discussed above. Table 2 shows the surface roughness and adsorbed amounts obtained from each fit to the Gaussian model described above.

<u>Alginate Used</u>	<u>Parameter</u>	<u>Bare surface</u>	<u>0.5 ppm</u>	<u>1 ppm</u>	<u>2.5 ppm</u>
MVM	Surface Roughness (\AA)	9.4 ± 0.3	9.4 ± 1.1	10.6 ± 2.0	16.1 ± 0.9
	Adsorbed amount (mg m^{-2})	-	1.0 ± 0.5	1.4 ± 0.1	1.6 ± 0.4
MVG	Surface Roughness (\AA)	8.9 ± 0.4	12.5 ± 1.3	18.0 ± 1.4	34.5 ± 0.7
	Adsorbed amount (mg m^{-2})	-	1.7 ± 0.3	2.7 ± 0.2	2.8 ± 0.3
MVG + 12 mM MgCl_2	Surface Roughness (\AA)	8.5 ± 0.6	-	-	27.3 ± 0.5
	Adsorbed amount (mg m^{-2})	-	-	-	0.7 ± 0.3

Table 2: Values for roughness and adsorbed amount obtained from fits to the data of the adsorption to the calcite surface of sodium alginate in pure water.

Figure 3b shows the experimental data measured for 2.5 ppm MVG in all three water contrasts. The data was fitted to a Gaussian layer model, as above, and again found to give good agreement in all datasets. The SLD of the H_2O contrast was again found to be higher than literature ($3.28 \pm 0.07 \times 10^{-6} \text{ \AA}^{-2}$) as for MVM alginate. The polymer distribution was found to extend further into solution compared to MVM, suggesting that the MVG molecule is less attracted to the calcite surface. This may also be related to a greater degree of cross-linking by calcium ions compared

1 to MVM alginate.¹⁷ The modelled roughness, $34.5 \pm 0.7 \text{ \AA}$, is large and the reflected signal
(above the critical edge) was much lower than for other measurements and reached background
at lower Q. The larger roughness indicates more calcium is released into the solution to achieve
equilibrium when compared to MVM alginate. The amount adsorbed with concentration follows
a similar profile as the MVM alginate; however, the value at 2.5 ppm is higher ($2.8 \pm 0.3 \text{ mg m}^{-2}$)
showing that more alginate is adsorbed when the polymer contains higher ratios of guluronate
residues.

HIGH GULUORNATE ALGINATE WITH MAGNESIUM

The reflectivity study of the MVG alginate adsorption to calcite in pure water indicated
significant surface roughening, attributed here to sequestration of calcium by the alginate
molecule. It is not possible to pre-saturate the water with calcium ions due to the insolubility of
calcium alginate and hence transport of calcium alginate to the calcite surface for adsorption
would be impossible. By adding 12 mM MgCl_2 (same concentration of calcium in saturated
 CaCO_3 water) and allowing the system to equilibrate, the magnesium ions bind non-specifically
with the alginate, reducing the effective charge of the polymer, making it less likely to be
attracted to the calcite surface.²³ The affinity of magnesium compared to calcium is thought to be
similar for MM and MG blocks but considerably smaller for GG blocks where specific
interactions with calcium prevail. As the alginates used in the study contain a mixture of all
blocks, we expect that the number of calcium ions removed from the surface will be lowered by
the presence of magnesium and therefore less roughening of the calcite surface upon introduction
to the sample cell. Magnesium ions have also been shown to bind to high energy sites on the
calcite surface inhibiting dissolution.³⁵

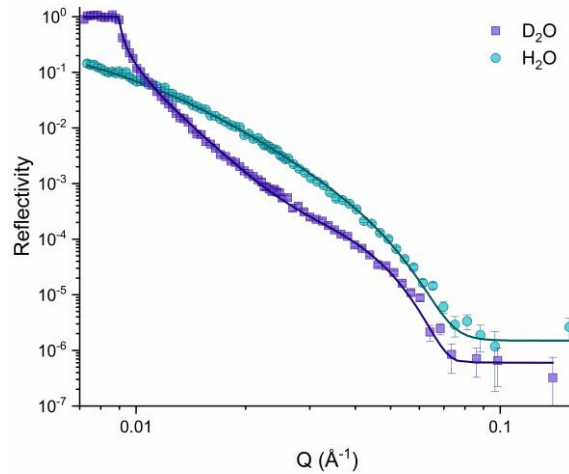


Figure 4: Experimental neutron reflectivity data of the calcite/water interface in the presence of 2.5 ppm MVG pre-equilibrated with 12 mM MgCl_2 in two contrasts, D_2O (purple squares) and H_2O (green circles). Calculated fits to the data are overlaid as solid lines.

Figure 4 shows the fitted reflectivity data upon introduction of 2.5 ppm MVG pre-equilibrated with 12 mM MgCl_2 in D_2O (purple circles) and H_2O (green circles). The corresponding SLD profile is provided in the supporting information (Figure S3). It was not possible to measure the CMC contrast due to time constraints of the experiment. The reflectivity profile shows a weak Kiessig fringe at higher Q suggesting a more defined adsorbed layer. Upon fitting the datasets, the maximum of the Gaussian polymer distribution is found to lie away from the calcite surface (Figure 5a) and the adsorbed amount is lower than for MVG in pure water (0.7 ± 0.3 compared to $2.8 \pm 0.3 \text{ mg m}^{-2}$). The surface roughness was also found to be lower $27.3 \pm 0.5 \text{ \AA}$ compared to $34.5 \pm 0.7 \text{ \AA}$. The reduction in surface roughness by pre-saturation of the MVG with 12 mM MgCl_2 was not as pronounced as expected, suggesting that the affinity of MVG alginate for calcium ions is high even if the charge is compensated by other divalent ions. The composition of the alginate (i.e. the proportion and order of M to G residues) plays a greater role in the extent of calcite surface roughening than the availability of divalent ions in the solution.

COMPARISON

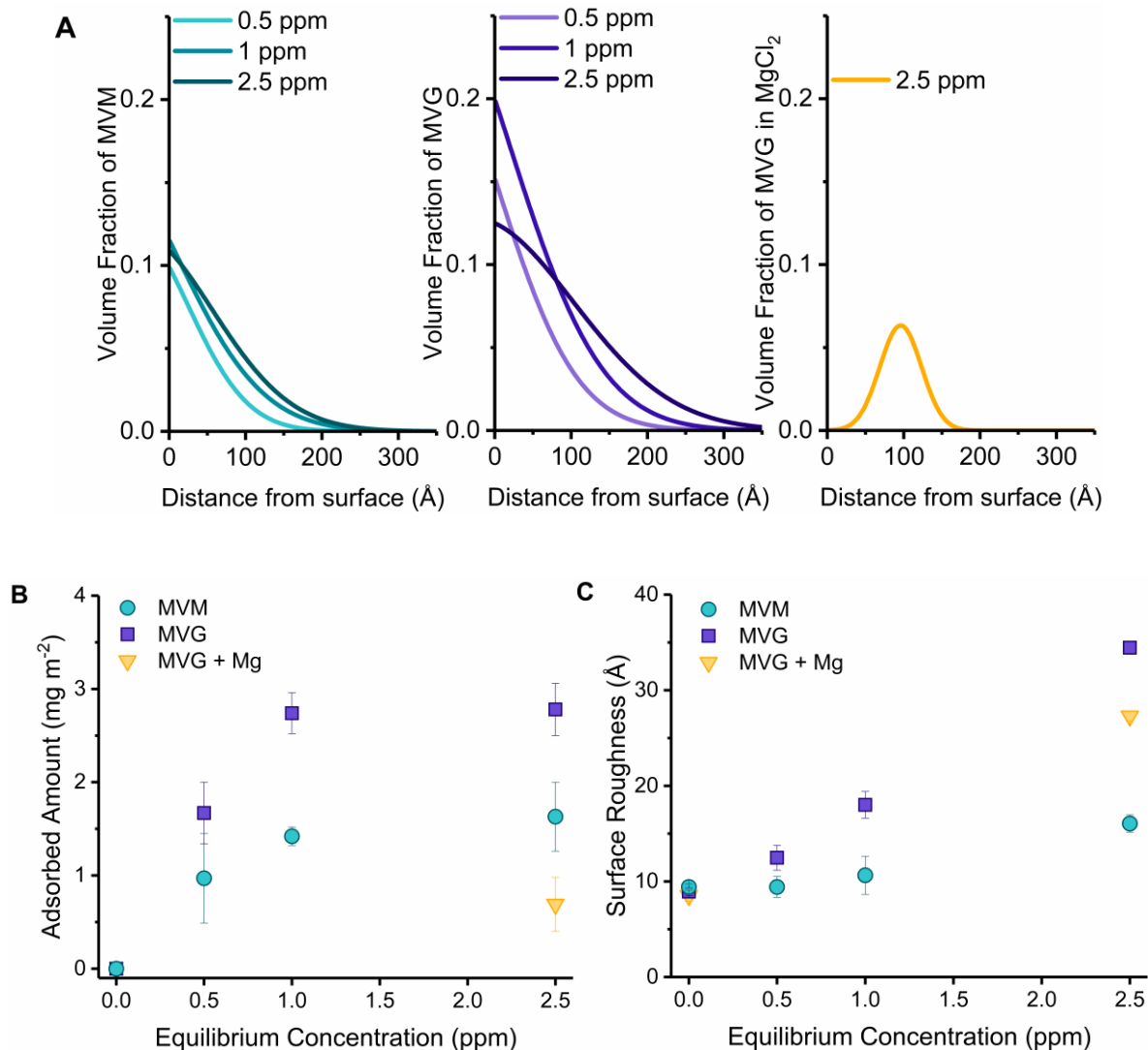


Figure 5: Comparison of the fitted results obtained from neutron fitting from sodium alginate adsorption to calcite surfaces. Data from MVM are shown as green circles, MVG as purple squares and MVG pre-equilibrated with 12mM MgCl_2 as yellow triangles. A. Gaussian distribution curves obtained from neutron reflection data fitting. B. Adsorption isotherm of sodium alginate to as calculated from the area under the curve of the Gaussian distribution of polymer adsorbed to the surface of calcite. C. Calcite surface roughness as a function of alginate concentration as obtained from neutron reflection fitting.

The data above shows the pronounced effect of the structural composition of sodium alginate on both the adsorption to and roughening of the surface of calcite crystals. Figure 5 summarizes the differences observed in adsorbed amounts (Fig 5b) and calcite surface roughness (Fig 5c). As described above the adsorbed amount increases from zero but reached a plateau at higher concentrations. The surface roughness increases with concentration, suggesting that the sequestering of calcium ions to the alginate drives the surface dissolution of calcite. In the reflection experiments reported here we must carefully consider the solution and surface behaviour. Sodium alginate in the presence of calcium ions readily converts to calcium alginate, which has a very low solubility. In order to introduce the alginate to the sample cell without precipitation the reflectivity experiments must be run in pure water. However, the introduction of pure water to the calcite surface increases surface dissolution and roughening.²⁶ The technique of neutron reflection requires very flat surfaces for a good reflected signal and hence any dissolution should be kept small. However, we also exploit the sensitivity of the reflection to this roughening as an ideal way of probing the surface dissolution. In previous studies, calcite in contact with pure water was found to roughen quickly before plateauing at a roughness 2 nm greater than the original surface.^{26,36}

DISCUSSION

This work is able to report on significant differences in the adsorption and surface roughening between the two investigated alginates: the alginate with the higher proportion of guluronate residues (MVG) is found increase calcite dissolution and shows a higher adsorption to the calcite surface compared to the alginate with a lower proportion of guluronate (MVM). As discussed above, alginate in the presence of calcium is reported to crosslink preferentially between GG units causing gelation and insolubility known as the ‘egg-box’ model.^{17,21} Therefore, the higher

1 the proportion of G residues the higher affinity for the calcium either at the surface or in solution.
2 However, the difference in M:G ratios between the two alginates is not large (46 % vs. 69%
3 guluronate residues) compared to the observed effects. The overall composition of the alginate
4 does not tell us the sequence of sugar residues, which can consist of three different repeat unit
5 sections, GG blocks, MM blocks or alternating MG blocks. The two alginates used in this study
6 have not been tested for the proportion of each of these pair wise sections. Therefore, the MVG
7 alginate may have a significantly higher proportion of the G residues in blocks of pure
8 guluronate (i.e. GG) rather than in alternating MG sections. GG blocks are the most effective at
9 crosslinking to form the 'egg box' structure that causes the molecule to become insoluble and
10 inflexible. Furthermore, higher proportions of G blocks may favour polymer-polymer
11 conformations rather than polymer-surface interactions and a more extended polymer
12 conformation reaching out from the surface as we see for the 2.5 ppm MVG alginate where the
13 polymer extends much farther out into solution as compared to MVM.

14 Addition of magnesium ions to the alginate solutions before introduction to the cell was found to
15 reduce the alginate binding and surface roughness. This effect is probably due to lower effective
16 charge of the alginate molecule by non-specific interactions with magnesium ions and lower
17 binding of calcium leading to less ion removal from the calcite surface.²² . In addition, the pre-
18 equilibrated alginate formed a different adsorption profile with maximum adsorbed amount lying
19 away from the surface as compared to that obtained from alginate adsorbed from pure water
20 solutions where the maximum was found to lie at the surface of the calcite.

21 Polyelectrolyte adsorption to oppositely charged surfaces in low ionic strength solutions is
22 complex and depends on a number of factors, including effective charge of the molecule due to
23 specific ion binding, ionic strength, and surface charge.^{37,38} At the interface, polyelectrolytes can

1 form trains, loops, and tails, in differing ratios depending on the system of study. For lower
2 polyelectrolyte charge densities, as would be expected for calcium saturated alginate in low salt
3 conditions, loop and tail adsorption is favoured.^{37,39} Entropic effects are also expected to play a
4 major role in the adsorption of oppositely charged polyelectrolyte adsorption through the release
5 of surface bound charge compensating counterions.⁴⁰ Also, in low ionic strength conditions the
6 electrical double layer would extend far enough to trap molecules electrostatically.

7 There is a large body of work attempting to describe the interactions between alginate and
8 calcium and its effect on calcium carbonate biomineralisation and dissolution. Experimental and
9 theoretical treatments of the 'egg box' model have shown the process to occur via three stages
10 during which mono then bi-dentate binding is seen before larger aggregations form, linked by
11 calcium ions.^{15,41} Borgogna *et. al.* reported the specific binding of calcium ions to alginate occurs
12 even at low concentration of calcium equilibrated with water.⁴² The binding proceeds via a tilted
13 egg-box model to minimise the repulsive interactions of the negative carboxylic acid groups.
14 Experiments at the interface are more scarce, Perry *et. al.* studied the dissolution behaviour of
15 calcite in the presence of alginate and reported that etch pits on the surface were lengthened
16 along the obtuse angle of the rhombohedral pit due to bidentate binding of the high energy
17 calcium at the edge of the pit to a GG dimer.^{43,44} In these experiments, the proportion of
18 guluronate residues is suggested to be the key to the increased interactions with calcium ions.

19 The present work agrees well with the solution and adsorption data from previous studies where
20 the high guluronate alginate (MVG) both adsorbs more to the calcite surface and dissolves the
21 surface to a greater extent than the same concentration of alginate with a lower guluronate
22 content (MVM). By studying this complex system using neutron reflection we were able to study
23 not only the adsorbed amount but also how the alginate adsorbed structure is affected by sugar

chain composition and the addition of magnesium. We find that the adsorbed amount is greatly reduced when studying bulk isotherms that have been centrifuged, indicating that 90% of the adsorbed polymer in the static system is only weakly attached to the surface. We also find that higher concentrations of MVG, with and without magnesium ions, extend further out into solution compared to low concentrations and low guluronate alginates suggesting more loop and tail formation and less affinity for the calcite surface. These results can be used to understand the formation of highly charged polysaccharide coatings and hydrogels in the presence of divalent cations for biodegradable tissue scaffolds or injectable biomaterials.⁴⁵⁻⁴⁷ Future studies in marine-like salt conditions as well as more complex extracellular matrix polysaccharides would build on this knowledge to understand how bacterial anchoring occurs to limestone rocks and soils.

CONCLUSION

Neutron reflectivity was able to demonstrate processes occurring during adsorption of alginate to calcite surfaces of interest to studies of bacterial anchoring to limestone rocks and biomineralisation. The system is extremely complex in terms of adsorption, solution chemistry and ion complexation. However, by careful control of the solution composition we are able to identify that these polymers are observed to form thick, highly hydrated layers at the surface. The dissolution of the calcite crystal due to polymer complexation was significant and was followed on an Ångström scale without perturbing or removing the adsorbed molecules. The conformation of the 1,4- α linkage of the polysaccharide was found to alter the adsorbed amount and structure of the molecule at the interface. Alginate molecules containing high proportions of guluronate sugar residues adsorbed more and removed more calcium ions from the calcite surface when compared to alginate with a lower proportion of guluronate. This work shows the

1 importance of polymer conformation and ion affinity on adsorption to dynamic dissolving calcite
2 interfaces.

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9 **Author Contributions**

10 KLB, INS, and SMC designed the study. All authors were involved in performing the
11 experiments and in the interpretation of the experimental results. The manuscript was written
12 through contributions of all authors who have approved the final version of the manuscript.

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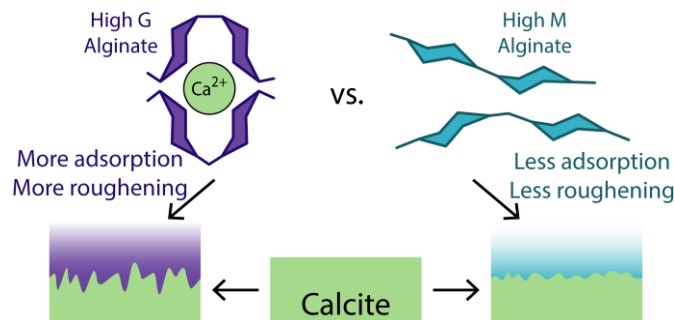
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The effect of alginate composition on adsorption to calcium carbonate surfaces

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ABSTRACT

Bacterial anchoring to limestone rocks is thought to occur by selective adsorption of biomolecules found in the extracellular matrix, such as polysaccharides. Here we study the adsorbed structure of a model matrix polysaccharide, sodium alginate, at the calcite/water interface using neutron reflection (NR). Sodium alginate was found to form highly hydrated layers extending up to 350 Å into solution at concentrations up to 2.5 ppm (the inflection point of the adsorption isotherm). The adsorption of alginate was driven by dissolution of the calcite surface through complexation of free calcium ions. This was shown using two alginates with differing ratios of sugar residues. Alginates with a higher proportion of guluronic acid (G) have a higher affinity for calcium ions and were found to cause the surface to dissolve to a greater extent and to adsorb more at the surface when compared to alginates with a higher proportion of

mannuronic acid (M). Adding magnesium to the high G alginate solution reduced dissolution of the surface and the adsorbed amount. In this work, we have shown that polysaccharide adsorption to sparingly soluble calcite interfaces is closely related to polymer conformation and affinity for free calcium ions in solution.

KEYWORDS

- Calcium Carbonate
- Calcite
- Biomineralization
- Alginate
- Polysaccharides
- Mannuronic Acid
- Guluronic Acid
- Adsorption
- Neutron Reflection
- Interfaces

INTRODUCTION

Most of the Earth's calcite (CaCO_3) is of biogenic origin from carbonaceous marine organism sedimentation. Calcium carbonate biomineralisation provides structure throughout Nature from avian eggshells to marine molluscs and coccoliths.^{1,2} The fine control of crystal morphology, phase and grain size is moderated through the production of various biomolecules, which also affect the mechanical properties.^{2,3} The precise mechanism by which these molecules interact with the crystallizing medium through nucleation and growth is an area of current interest in several areas, including amorphous calcium carbonate precursor phases (ACC).³⁻⁹

Calcium carbonate can be precipitated in several different polymorphs of varying stability. The most stable polymorph under ambient conditions is calcite; it can form large defect-free crystals, making it suitable as the focus of this experimental study. It is sparingly soluble in water with a solubility product at room temperature of $K_{\text{sp}} = 3.7 \times 10^{-9} \text{ mol dm}^{-3}$.¹⁰ This dissolution complicates the analysis of adsorption to calcite surfaces as acidic conditions can lead to a highly

dynamic interface. The dissolution or precipitation of calcite is closely linked with other aqueous equilibria that determine the extent of dissolution of the surface.¹¹

The driving force for adsorption of polyanionic polysaccharides is expected to be the affinity of the charged carboxylate groups for calcium on the positively charged calcite surface. Calcium cations released from the calcite surface are sequestered to the sugar forming a complex. This process in turn removes calcium from the solution shifting the equilibrium such that more calcium carbonate solid is dissolved. Polyanionic polysaccharides have been shown to affect the crystal growth structure of calcium carbonate by interacting with the mineral surface suggesting specific interactions with the calcite surface.¹² Furthermore, negatively charged sugars secreted by bacteria into the extracellular matrix may aid anchoring of the cell to calcitic limestone rocks in marine environments.^{13,14}

Sodium alginate has been chosen for this study as a model polysaccharide due to its well-understood structural characteristics and calcium binding properties, allowing the interplay between complex-mediated surface dissolution and polymer adsorption to be investigated.¹⁵

Alginates are a major structural component of marine algae and secreted by some bacteria, most notably *Pseudomonas aeruginosa* and *Azotobacter vinelandii*.¹⁶ As schematically illustrated in Figure 1, sodium alginate is a copolymer of two epimers, mannuronic acid (M) and guluronic acid (G), which differ only by the conformation of the 1,4 glycosidic link. The two sugar residues can be found either as homopolymeric blocks of each sugar or as alternating MG sequences.¹⁷ The primary structure of alginate affects the macromolecular properties greatly. GG blocks are rigid as the axial linkage decreases movement and provides a binding site for divalent cations. Conversely, MM blocks are joined via an equatorial glycosidic linkage are therefore

relatively free.¹⁷ The molecular conformation of the pairs, MM, MG and GG are included in Figure 1.

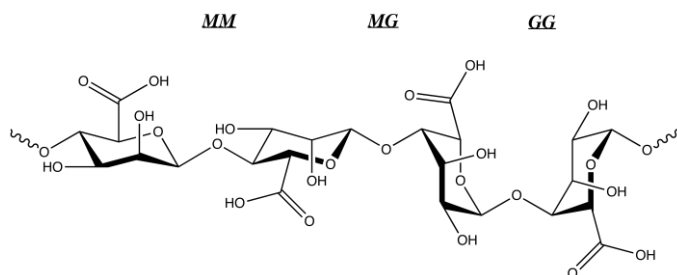


Figure 1: Molecular structure of the alginic acid showing the various links between the two epimers, MM, MG and GG diblocks.

Two gram negative, alginate producing, bacteria, *Pseudomonas aeruginosa* and *Azotobacter vinelandii*, secrete alginates with different compositions, macromolecular properties and functions. *P. aeruginosa* secretes alginate as a protective envelope and adhesive molecule in biofilm formation in the lung, these alginates have little to no G blocks.¹⁶ Conversely, *A. vinelandii*, a soil bacterium that forms exopolysaccharide cysts in soil to protect from mechanical stress and desiccation during dormancy, produces alginates with high ratios of GG blocks and alternating sequences in the outer protective layer.¹⁶

The more rigid GG sections provide well-defined chelation sites, which leads to gelation on addition of calcium. The more flexible MM and MG blocks deform to allow adjacent GG disaccharide units to line up form a chelating cage composed of four G saccharide units.¹⁸ This ability to co-ordinate is strongly linked to conformational structure, alginates with a higher proportion of G (and G blocks) gel at lower concentrations of calcium ions and form stronger and more brittle gels.^{19,20} Grant *et al.* defined this guluronate-Ca interaction the ‘egg-box model’ due to the alignment of guluronate chains along a plane of calcium ions.^{17,21} In contrast to

1 calcium, the binding of magnesium with alginate molecules is reported to be a non-specific
2 interaction.^{22,23} Magnesium alginate is soluble and does not strongly interact with any specific
3 binding sites.^{23,24}

4 In this study we have used neutron reflection and bulk solution depletion isotherms to compare
5 the adsorption of two different sodium alginate samples with either a low (MVM) or high
6 (MVG) content of guluronate residues to calcium carbonate surfaces. Considering the higher
7 affinity of guluronate residues to calcium ions in solution, the two alginates are expected to show
8 very different surface adsorption profiles in terms of both adsorbed amount and structure.
9 Neutron reflectivity allows the measurement of complex adsorbed structures at the solid/liquid
10 interface, something which is challenging using other techniques. The study of the solid/liquid
11 interface using neutron reflection has been reasonably limited to a small number of single crystal
12 interfaces, mainly silicon, quartz and sapphire.²⁵ Recently the number of mineral surfaces used in
13 reflection experiments has widened to include calcite, however, previous work has focused on
14 the adsorption of industrial chemicals and surfactants at the calcite/liquid interface.^{26–28} Here we
15 aim to investigate the adsorption of biopolymers to calcite surfaces in aqueous environments to
16 show how polymer conformation and calcium ion dissolution from the calcite surface can affect
17 adsorbed amount and structure at the interface.

18 EXPERIMENTAL

19 MATERIALS

20 All experiments were carried out using two ultrapure and well-characterised alginates (Pronova
21 UP Test kit) obtained from Novamatrix. The two alginates tested were of medium viscosity
22 (>200 mPa·s) and high molecular weight (>200 kDa) and differ by the ratio of the two sugar

1 epimers. The high guluronate sample (Medium Viscosity Guluronate, MVG) was reported to
2 have a viscosity of 263 mPa's and contain 69 % guluronate residues. The high mannuronate
3 sample (Medium Viscosity Mannuronate, MVM) was found to have a higher viscosity (553
4 mPa's) with a mannuronic acid content of 54 %. All values quoted here were obtained from the
5 manufacturers certificate of analysis of the batch used.

6 Calcium carbonate powder ($\geq 99\%$) used for adsorption isotherms and to saturate water for NR
7 experiments was purchased from Sigma Aldrich. Calcite crystals used in neutron reflection
8 experiments were optically clear Iceland Spar crystals obtained from P&E Export Ltd, Brazil.
9 The large rhombohedral crystals were cut to a cuboid shape (40 x 55 x 15 mm) and polished to a
10 low roughness by Crystran, Poole, UK. The surface for reflectivity was the most stable $\{10\bar{1}4\}$
11 face, the crystallographic plane found at the surface of the natural rhomb. Attempts were made to
12 cleave the surface rather than polish, however, it was not possible to obtain an area large enough
13 for reflectivity studies (roughly 20 cm²) without introducing large steps, causing the cells to leak.
14 The roughness of the polished crystals was assessed by X-ray reflectivity on a Rigaku SmartLab
15 X-ray diffractometer (ISIS, Rutherford Appleton Laboratory, Didcot, UK). This measurement
16 gave an approximate indication of the quality of the surface (data shown in supporting
17 information, Figure S1). Crystals, which had an X-ray roughness less than 15 Å, were then used
18 in neutron reflectivity experiments.

19 ADSORPTION ISOTHERMS

20 Isotherm measurements were carried out using a solution depletion batch method. 2g of calcium
21 carbonate powder was homogenised with 498g ultrapure water using a Silverson L4R shear
22 mixer for 10 minutes. The slurry was then weighed into centrifuge tubes and increasing amounts

of alginate stock solution was added. The samples were agitated overnight and centrifuged at 15,000 G for 20 minutes. The supernatant was removed and filtered through a 0.45 µm polyethersulfone syringe filter (QMX, UK). The Total Organic Carbon content of the supernatant was measured using a GE InnovOx TOC Analyser. During analysis, the supernatant was acidified and purged with nitrogen to remove traces of inorganic carbonates. The concentration of non-purgeable carbon remaining in the supernatant was then used to calculate the adsorbed amount of alginate.

NEUTRON REFLECTIVITY

The experiment is performed by illuminating a large flat crystal with a collimated beam of neutrons, which reflect and/or refract at the solid/liquid interface. The intensity of the reflected beam is then measured as a function of wavelength and reflected angle. In this experiment, the D17 reflectometer at the Institut Laue-Langevin, France,²⁹ was used in the time-of flight (TOF) mode where a polychromatic beam of neutrons is directed at the sample and the wavelength determined by the time taken for neutrons to reach the detector from the source. The determined wavelength values are converted to a momentum transfer vector, Q , calculated from equation 1.

$$Q = \frac{4\pi \sin\theta}{\lambda} \quad 1$$

The use of the TOF method on D17 allows the entire scattering range of interest of the reflectivity curve to be measured using only two incident angles, 0.6° and 2.0°, to cover the Q range of interest (0.006-0.2 Å⁻¹). To ensure no contribution from the edges of the sample, a constant beam footprint of 30 x 45 mm² was maintained on the sample surface. The dQ/Q resolution varied between 1.5-10% over the Q range measured, during data fitting a value of 4% resolution was used. The data reduction was performed using the COSMOS software.³⁰

The calcite crystals were cleaned by UV-Ozone treatment (BioForce Nano Procleaner). After irradiation, a small drop of filtered calcium carbonate saturated water was placed on the surface to assess wettability. If the droplet spread the crystal was considered clean, placed immediately against a Teflon trough and sealed in a neutron reflectivity cell (details of cell reflection sample cell are given in Stocker *et. al.*²⁶). Sample changes were carried out by draining the sample cell by pipette (3 mL cell volume) and refilling in-situ with the required solution, this process was repeated three times and the cell sealed with Teflon plugs.

ANALYSIS OF REFLECTIVITY DATA

The shape of the reflectivity curve at a single interface is determined by the surface roughness and the change in neutron refractive index upon moving from one medium to the next through the surface. Neutron reflection theory is outlined elsewhere.³¹ A particularly powerful aspect of neutron reflection is the seemingly random nature of neutron scattering lengths across the periodic table and between isotopes. The scattering length density (SLD) of a material is calculated using equation 2 where N is the number density of atoms in a material and b_i is the scattering length of each nucleus.

$$\rho = \sum N b_i \quad 2$$

The isotope effect is most clearly seen by the very different SLDs of H₂O and D₂O, $-0.56 \times 10^{-6} \text{ \AA}^{-2}$ and $6.35 \times 10^{-6} \text{ \AA}^{-2}$, respectively. Using isotopic substitution it is possible to study different reflectivity profiles in the same chemical environment. In this work, we have measured reflectivity profiles of the calcite/alginate solution in H₂O, D₂O, and a mixture of the two to form a solution that has the same scattering length density of the calcite crystal ($4.69 \times 10^{-6} \text{ \AA}^{-2}$). This combination of solution contrasts is important to constrain the structural model. By using a

1 solution with the same SLD as the calcite, a reflected signal is only seen from the adsorbed
2 alginate (alginate SLD= $2.6 \times 10^{-6} \text{ \AA}^{-2}$, calculated from the expected molecular formula and
3 specific gravity).

4 The measured reflectivity profile is then analysed by comparison of the experimental data with a
5 model structure. The general approach is to constrain the structural model as much as possible to
6 fit the data. If a single layer is found not be sufficient to fit the experimental data, adsorbed
7 layers can be broken into several smaller blocks/layers to describe adsorbed structures in more
8 detail. In some cases a mathematical description of the SLD profile is appropriate, such as
9 exponential or Gaussian.³² In all of these cases, the total reflected intensity from adsorbed layers
10 is calculated using the matrix method developed by Abeles and used in reflectivity fitting
11 programs such as RasCAL.³³

12 The polymer segment density distribution normal to the surface was modelled in several ways,
13 such as discrete layers, exponential decrease from the surface, or a Gaussian distribution. The
14 best fit to the data in all cases was found using the Gaussian distribution model. The SLD profile
15 through the interface was modelled by dividing a 300 Å region into 10 Å sections with an
16 interlayer roughness fixed to 5 Å to smooth out each interface. The volume fraction of alginate
17 was then calculated in each section and converted to an SLD by assuming the rest of the layer
18 was filled with the subphase water. In all adsorbed alginate datasets it was observed that the
19 volume fraction of alginate had reached background well before the model cut off at 300 Å
20 except for the 2.5 ppm MVG where the model had to be extended to 350 Å to reach background.
21 Using the modelled volume fraction distribution, an adsorbed amount was obtained from the area
22 under the Gaussian polymer segment density distribution curve. Although it was possible to fit a
23 small variation in the Gaussian profile, in all cases the distribution was found to conserve the

area under the curve, within error, providing a good test of the robustness of the values for adsorbed amount. Errors were calculated using the bootstrap error analysis in the RasCAL program, where random parameter values are generated and a fit performed 100 times to generate confidence intervals. The parameter errors were then used to calculate the error in adsorbed amount, assuming the maximum and minimum possible values. The SLDs of substrate, subphase, and adsorbate were fixed, where possible, according to the literature values (Table S1). During the experiment it was noted that there was incomplete exchange of the liquids within the cells as the SLD of the H₂O subphase could not be fitted to the literature value, but rather a mixture of H₂O containing some D₂O, only when the liquid in the cell was exchanged from D₂O to H₂O in situ (despite repeated exchanges). This may be due to insufficient mixing of the two liquids due to the density difference in the cell and the high contact angle between the water and the hydrophobic surface of the Teflon trough.

RESULTS

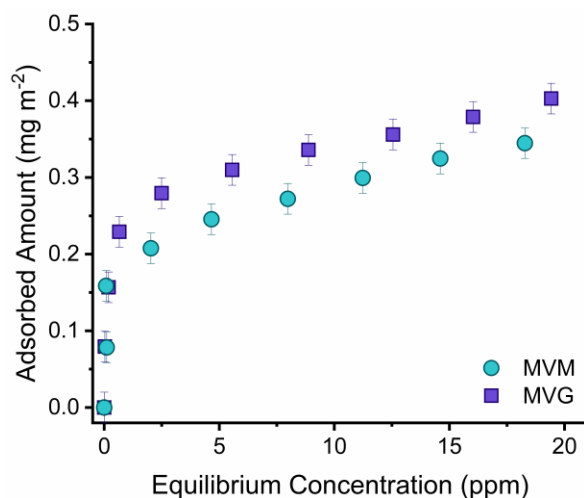


Figure 2: Isotherm data of sodium alginate adsorption to calcite powder in water as measured by total organic carbon analysis (TOC). MVM alginate is shown as green circles and MVG as purple squares.

Figure 2 presents bulk solution depletion isotherms for the adsorption of MVM (green circles) and MVG (purple squares) to calcium carbonate powder. It can be seen that there is a small difference between the two alginate solutions with MVG adsorbing more to the surface than MVM. At low concentrations, all alginate is adsorbed as seen from a steep initial increase in adsorbed amount before the turning point of the isotherm at an equilibrium concentrations of roughly 2.5 ppm. At higher concentrations, the slope becomes shallower but not flat suggesting that the adsorption does not form a monolayer at the surface. There may be some precipitation of the alginate at higher concentrations as the calcium is dissolved into solution forming insoluble calcium alginate. The interaction between the calcium carbonate surface and alginate is observed to be weak due to the low adsorbed amount at equilibrium. This effect could be accentuated by the sample preparation for TOC analysis. In order to remove calcium carbonate powder from the supernatant, the samples are centrifuged at 15,000 G for 20 minutes which is likely to shear off any loosely bound alginate from the surface leaving only the tightly bound molecules that are effectively irreversibly adsorbed by attachment at multiple points.

NEUTRON REFLECTION

Each calcite crystal was first measured in three saturated calcium carbonate water contrasts, D₂O, H₂O, and water contrast matched to calcite ($CMC = 4.69 \times 10^{-6} \text{Å}^{-2}$). A representative dataset is shown in the supporting information (Figure S2). Previous experiments have shown that the surface roughness of calcite does not increase over time in the presence of water saturated with calcium carbonate powder.²⁷ The surface cleanliness was confirmed by the absence of any reflected signal above the background level in the CMC contrast (not shown). Measuring the reflectivity in contrast matched water is especially useful due to the high sensitivity to adsorbed layers at the surface. Unlike silicon crystals, which always have a surface

oxide layer and always show a signal in contrast matched water, if the surface of calcite is completely clean there will be no signal recorded. The two (D_2O and H_2O) data sets were fitted simultaneously using only two fitting variables, surface roughness and H_2O contrast. All bare calcite crystals for the study were successfully fitted to a single interface model and the surface roughness varied from $8.5 \pm 0.6 \text{ \AA}$ to $9.4 \pm 0.3 \text{ \AA}$.

Before adsorption measurements using neutron reflection it is important to completely characterise the surface that the adsorbate will bind to. Unlike many other surfaces used in reflection the calcite surface can be dynamic and of biogenic origin. Measurements of the bare surface are therefore useful to assess the cleanliness of the substrate and provide an initial roughness characterisation. When calcite is cleaved or polished, high-energy bonding sites are exposed. In air, these sites are likely to be contaminated with species from the atmosphere, however, this bonding of contaminants is generally weak and upon immersion, water molecules compete, removing the contaminants and hydrating the surface with pH sensitive groups.^{11,26,34}

ALGINATE ADSORPTION

Figure 3 presents experimental neutron reflectivity data of the calcite/water interface in the presence of (a) 2.5 ppm MVM and (b) 2.5 ppm MVG alginate in three contrasts, D_2O (purple squares), H_2O (green circles), and water contrast matched to calcite (CMC, yellow triangles). Corresponding SLD profiles are provided in the supporting information (Figure S3). Alginate solutions were introduced to the sample cells at concentrations of 0.5 and 1 ppm in D_2O . Three water contrasts were recorded for the highest concentration, 2.5 ppm. The concentration of 2.5 ppm was chosen to be just above the turning point of the plateau for both MVM and MVG

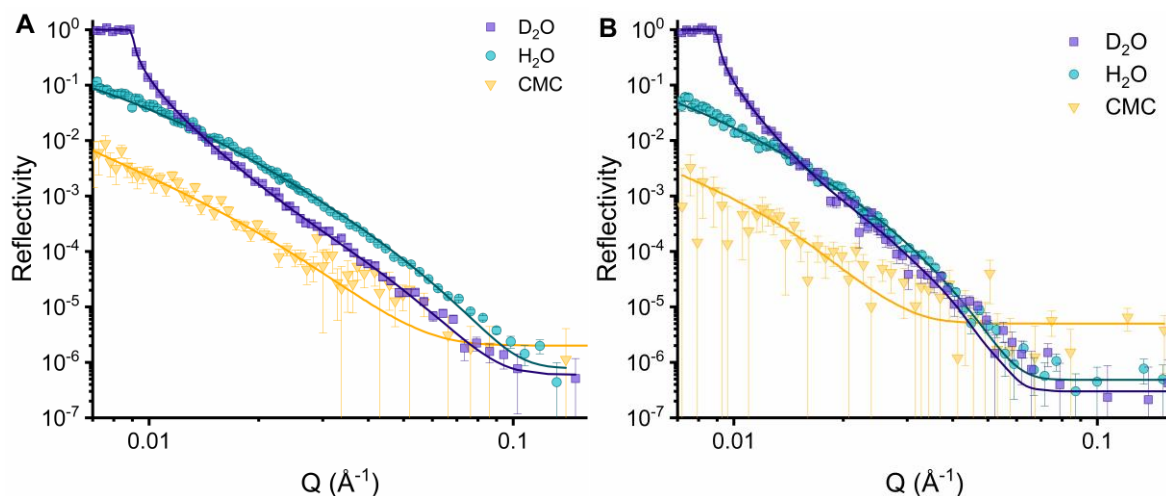


Figure 3: Experimental neutron reflectivity data of the calcite/water interface in the presence of **A** 2.5 ppm MVM and **B** 2.5 ppm MVG alginate in three contrasts, D₂O (purple squares), H₂O (green circles) and water contrast matched to calcite (CMC, yellow triangles). Calculated fits to the data are overlaid as solid lines.

Upon introduction of alginate the intensity of the reflectivity in both the H₂O and D₂O contrasts drop, suggesting that the surface is roughening and/or a diffuse layer is adsorbed. A signal is also seen in the contrast matched water indicating an adsorbed layer was present at the surface. This signal in the contrast matched water is purely from any adsorbed material at the interface and therefore has a rather low signal-to-noise ratio and large error bars. The 2.5 ppm MVM data was fitted to a Gaussian layer model with an adsorbed amount of $1.6 \pm 0.4 \text{ mg m}^{-2}$ and a surface roughness of $16.1 \pm 0.9 \text{ \AA}$. The volume fraction of alginate at the surface was found to be 0.098 showing that the adsorbed layer is highly hydrated at the interface. The volume fraction decreases away from the surface into solution before reaching zero between 200 and 250 \AA . As

the concentration of MVM alginate increases, the amount adsorbed at the surface initially increases and begins to level off reaching $1.6 \pm 0.4 \text{ mg m}^{-2}$ at 2.5 ppm MVM. The SLD of the subphase in the H_2O was fitted to be higher ($2.28 \pm 0.05 \times 10^{-6} \text{ \AA}^{-2}$) than the literature value of $0.56 \times 10^{-6} \text{ \AA}^{-2}$, due to incomplete exchange, as discussed above. Table 2 shows the surface roughness and adsorbed amounts obtained from each fit to the Gaussian model described above.

<u>Alginate Used</u>	<u>Parameter</u>	<u>Bare surface</u>	<u>0.5 ppm</u>	<u>1 ppm</u>	<u>2.5 ppm</u>
MVM	Surface Roughness (\AA)	9.4 ± 0.3	9.4 ± 1.1	10.6 ± 2.0	16.1 ± 0.9
	Adsorbed amount (mg m^{-2})	-	1.0 ± 0.5	1.4 ± 0.1	1.6 ± 0.4
MVG	Surface Roughness (\AA)	8.9 ± 0.4	12.5 ± 1.3	18.0 ± 1.4	34.5 ± 0.7
	Adsorbed amount (mg m^{-2})	-	1.7 ± 0.3	2.7 ± 0.2	2.8 ± 0.3
MVG + 12 mM MgCl_2	Surface Roughness (\AA)	8.5 ± 0.6	-	-	27.3 ± 0.5
	Adsorbed amount (mg m^{-2})	-	-	-	0.7 ± 0.3

Table 2: Values for roughness and adsorbed amount obtained from fits to the data of the adsorption to the calcite surface of sodium alginate in pure water.

Figure 3b shows the experimental data measured for 2.5 ppm MVG in all three water contrasts. The data was fitted to a Gaussian layer model, as above, and again found to give good agreement in all datasets. The SLD of the H_2O contrast was again found to be higher than literature ($3.28 \pm 0.07 \times 10^{-6} \text{ \AA}^{-2}$) as for MVM alginate. The polymer distribution was found to extend further into solution compared to MVM, suggesting that the MVG molecule is less attracted to the calcite surface. This may also be related to a greater degree of cross-linking by calcium ions compared

1 to MVM alginate.¹⁷ The modelled roughness, $34.5 \pm 0.7 \text{ \AA}$, is large and the reflected signal
(above the critical edge) was much lower than for other measurements and reached background
at lower Q. The larger roughness indicates more calcium is released into the solution to achieve
equilibrium when compared to MVM alginate. The amount adsorbed with concentration follows
a similar profile as the MVM alginate; however, the value at 2.5 ppm is higher ($2.8 \pm 0.3 \text{ mg m}^{-2}$)
showing that more alginate is adsorbed when the polymer contains higher ratios of guluronate
residues.

HIGH GULUORNATE ALGINATE WITH MAGNESIUM

The reflectivity study of the MVG alginate adsorption to calcite in pure water indicated
significant surface roughening, attributed here to sequestration of calcium by the alginate
molecule. It is not possible to pre-saturate the water with calcium ions due to the insolubility of
calcium alginate and hence transport of calcium alginate to the calcite surface for adsorption
would be impossible. By adding 12 mM MgCl_2 (same concentration of calcium in saturated
 CaCO_3 water) and allowing the system to equilibrate, the magnesium ions bind non-specifically
with the alginate, reducing the effective charge of the polymer, making it less likely to be
attracted to the calcite surface.²³ The affinity of magnesium compared to calcium is thought to be
similar for MM and MG blocks but considerably smaller for GG blocks where specific
interactions with calcium prevail. As the alginates used in the study contain a mixture of all
blocks, we expect that the number of calcium ions removed from the surface will be lowered by
the presence of magnesium and therefore less roughening of the calcite surface upon introduction
to the sample cell. Magnesium ions have also been shown to bind to high energy sites on the
calcite surface inhibiting dissolution.³⁵

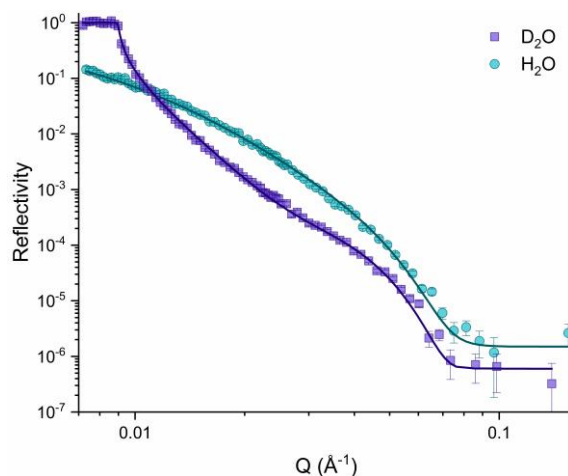


Figure 4: Experimental neutron reflectivity data of the calcite/water interface in the presence of 2.5 ppm MVG pre-equilibrated with 12 mM MgCl_2 in two contrasts, D_2O (purple squares) and H_2O (green circles). Calculated fits to the data are overlaid as solid lines.

Figure 4 shows the fitted reflectivity data upon introduction of 2.5 ppm MVG pre-equilibrated with 12 mM MgCl_2 in D_2O (purple circles) and H_2O (green circles). The corresponding SLD profile is provided in the supporting information (Figure S3). It was not possible to measure the CMC contrast due to time constraints of the experiment. The reflectivity profile shows a weak Kiessig fringe at higher Q suggesting a more defined adsorbed layer. Upon fitting the datasets, the maximum of the Gaussian polymer distribution is found to lie away from the calcite surface (Figure 5a) and the adsorbed amount is lower than for MVG in pure water (0.7 ± 0.3 compared to $2.8 \pm 0.3 \text{ mg m}^{-2}$). The surface roughness was also found to be lower $27.3 \pm 0.5 \text{ \AA}$ compared to $34.5 \pm 0.7 \text{ \AA}$. The reduction in surface roughness by pre-saturation of the MVG with 12 mM MgCl_2 was not as pronounced as expected, suggesting that the affinity of MVG alginate for calcium ions is high even if the charge is compensated by other divalent ions. The composition of the alginate (i.e. the proportion and order of M to G residues) plays a greater role in the extent of calcite surface roughening than the availability of divalent ions in the solution.

COMPARISON

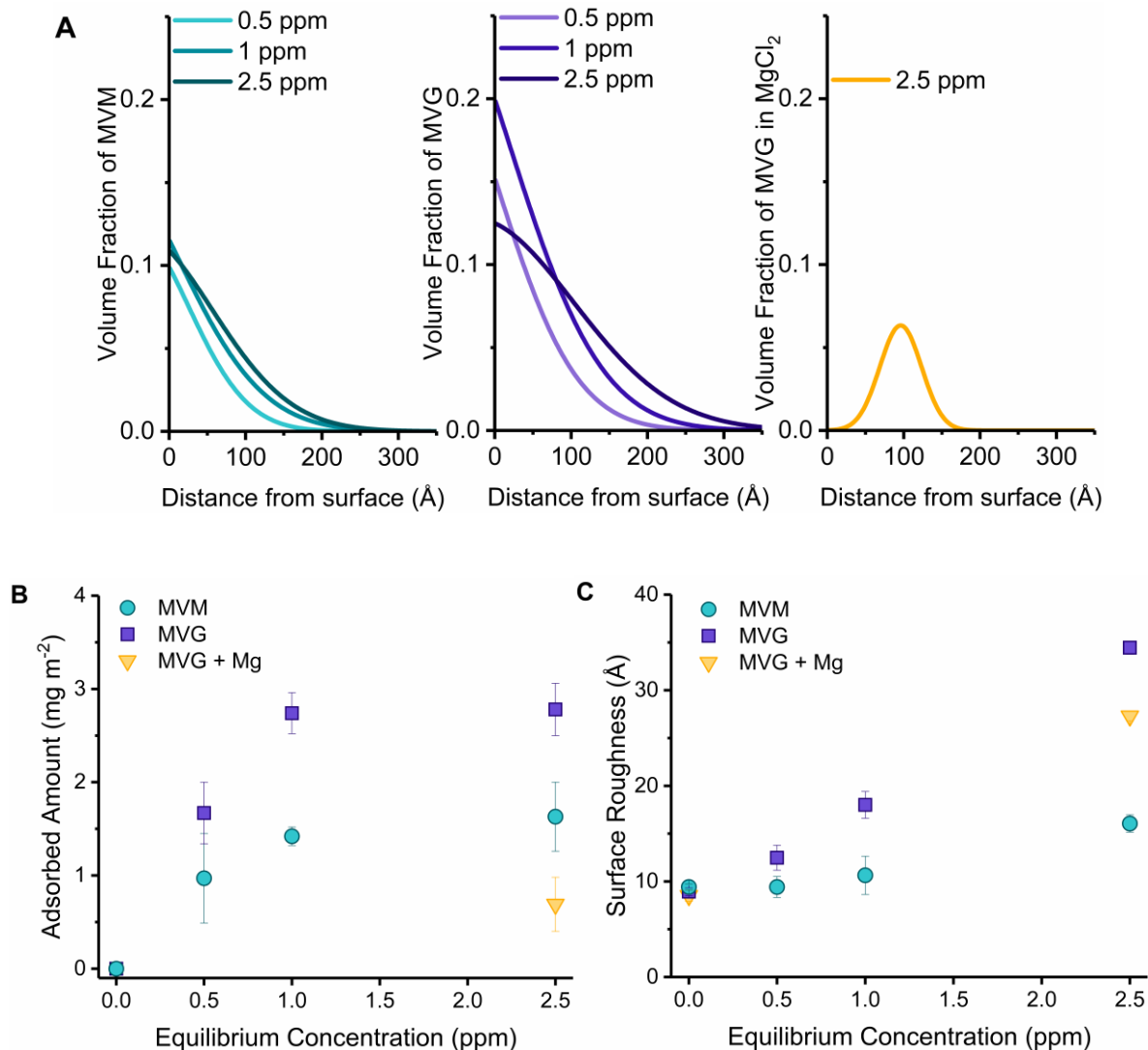


Figure 5: Comparison of the fitted results obtained from neutron fitting from sodium alginate adsorption to calcite surfaces. Data from MVM are shown as green circles, MVG as purple squares and MVG pre-equilibrated with 12mM MgCl_2 as yellow triangles. A. Gaussian distribution curves obtained from neutron reflection data fitting. B. Adsorption isotherm of sodium alginate to as calculated from the area under the curve of the Gaussian distribution of polymer adsorbed to the surface of calcite. C. Calcite surface roughness as a function of alginate concentration as obtained from neutron reflection fitting.

1 The data above shows the pronounced effect of the structural composition of sodium alginate on
2 both the adsorption to and roughening of the surface of calcite crystals. Figure 5 summarizes the
3 differences observed in adsorbed amounts (Fig 5b) and calcite surface roughness (Fig 5c). As
4 described above the adsorbed amount increases from zero but reached a plateau at higher
5 concentrations. The surface roughness increases with concentration, suggesting that the
6 sequestering of calcium ions to the alginate drives the surface dissolution of calcite. In the
7 reflection experiments reported here we must carefully consider the solution and surface
8 behaviour. Sodium alginate in the presence of calcium ions readily converts to calcium alginate,
9 which has a very low solubility. In order to introduce the alginate to the sample cell without
10 precipitation the reflectivity experiments must be run in pure water. However, the introduction of
11 pure water to the calcite surface increases surface dissolution and roughening.²⁶ The technique of
12 neutron reflection requires very flat surfaces for a good reflected signal and hence any
13 dissolution should be kept small. However, we also exploit the sensitivity of the reflection to this
14 roughening as an ideal way of probing the surface dissolution. In previous studies, calcite in
15 contact with pure water was found to roughen quickly before plateauing at a roughness 2 nm
16 greater than the original surface.^{26,36}

17 DISCUSSION

18 This work is able to report on significant differences in the adsorption and surface roughening
19 between the two investigated alginates: the alginate with the higher proportion of guluronate
20 residues (MVG) is found increase calcite dissolution and shows a higher adsorption to the calcite
21 surface compared to the alginate with a lower proportion of guluronate (MVM). As discussed
22 above, alginate in the presence of calcium is reported to crosslink preferentially between GG
23 units causing gelation and insolubility known as the ‘egg-box’ model.^{17,21} Therefore, the higher

1 the proportion of G residues the higher affinity for the calcium either at the surface or in solution.
2 However, the difference in M:G ratios between the two alginates is not large (46 % vs. 69%
3 guluronate residues) compared to the observed effects. The overall composition of the alginate
4 does not tell us the sequence of sugar residues, which can consist of three different repeat unit
5 sections, GG blocks, MM blocks or alternating MG blocks. The two alginates used in this study
6 have not been tested for the proportion of each of these pair wise sections. Therefore, the MVG
7 alginate may have a significantly higher proportion of the G residues in blocks of pure
8 guluronate (i.e. GG) rather than in alternating MG sections. GG blocks are the most effective at
9 crosslinking to form the 'egg box' structure that causes the molecule to become insoluble and
10 inflexible. Furthermore, higher proportions of G blocks may favour polymer-polymer
11 conformations rather than polymer-surface interactions and a more extended polymer
12 conformation reaching out from the surface as we see for the 2.5 ppm MVG alginate where the
13 polymer extends much farther out into solution as compared to MVM.

14 Addition of magnesium ions to the alginate solutions before introduction to the cell was found to
15 reduce the alginate binding and surface roughness. This effect is probably due to lower effective
16 charge of the alginate molecule by non-specific interactions with magnesium ions and lower
17 binding of calcium leading to less ion removal from the calcite surface.²² . In addition, the pre-
18 equilibrated alginate formed a different adsorption profile with maximum adsorbed amount lying
19 away from the surface as compared to that obtained from alginate adsorbed from pure water
20 solutions where the maximum was found to lie at the surface of the calcite.

21 Polyelectrolyte adsorption to oppositely charged surfaces in low ionic strength solutions is
22 complex and depends on a number of factors, including effective charge of the molecule due to
23 specific ion binding, ionic strength, and surface charge.^{37,38} At the interface, polyelectrolytes can

1 form trains, loops, and tails, in differing ratios depending on the system of study. For lower
2 polyelectrolyte charge densities, as would be expected for calcium saturated alginate in low salt
3 conditions, loop and tail adsorption is favoured.^{37,39} Entropic effects are also expected to play a
4 major role in the adsorption of oppositely charged polyelectrolyte adsorption through the release
5 of surface bound charge compensating counterions.⁴⁰ Also, in low ionic strength conditions the
6 electrical double layer would extend far enough to trap molecules electrostatically.

7 There is a large body of work attempting to describe the interactions between alginate and
8 calcium and its effect on calcium carbonate biomineralisation and dissolution. Experimental and
9 theoretical treatments of the 'egg box' model have shown the process to occur via three stages
10 during which mono then bi-dentate binding is seen before larger aggregations form, linked by
11 calcium ions.^{15,41} Borgogna *et. al.* reported the specific binding of calcium ions to alginate occurs
12 even at low concentration of calcium equilibrated with water.⁴² The binding proceeds via a tilted
13 egg-box model to minimise the repulsive interactions of the negative carboxylic acid groups.
14 Experiments at the interface are more scarce, Perry *et. al.* studied the dissolution behaviour of
15 calcite in the presence of alginate and reported that etch pits on the surface were lengthened
16 along the obtuse angle of the rhombohedral pit due to bidentate binding of the high energy
17 calcium at the edge of the pit to a GG dimer.^{43,44} In these experiments, the proportion of
18 guluronate residues is suggested to be the key to the increased interactions with calcium ions.

19 The present work agrees well with the solution and adsorption data from previous studies where
20 the high guluronate alginate (MVG) both adsorbs more to the calcite surface and dissolves the
21 surface to a greater extent than the same concentration of alginate with a lower guluronate
22 content (MVM). By studying this complex system using neutron reflection we were able to study
23 not only the adsorbed amount but also how the alginate adsorbed structure is affected by sugar

chain composition and the addition of magnesium. We find that the adsorbed amount is greatly reduced when studying bulk isotherms that have been centrifuged, indicating that 90% of the adsorbed polymer in the static system is only weakly attached to the surface. We also find that higher concentrations of MVG, with and without magnesium ions, extend further out into solution compared to low concentrations and low guluronate alginates suggesting more loop and tail formation and less affinity for the calcite surface. These results can be used to understand the formation of highly charged polysaccharide coatings and hydrogels in the presence of divalent cations for biodegradable tissue scaffolds or injectable biomaterials.^{45–47} Future studies in marine-like salt conditions as well as more complex extracellular matrix polysaccharides would build on this knowledge to understand how bacterial anchoring occurs to limestone rocks and soils.

CONCLUSION

Neutron reflectivity was able to demonstrate processes occurring during adsorption of alginate to calcite surfaces of interest to studies of bacterial anchoring to limestone rocks and biomineralisation. The system is extremely complex in terms of adsorption, solution chemistry and ion complexation. However, by careful control of the solution composition we are able to identify that these polymers are observed to form thick, highly hydrated layers at the surface. The dissolution of the calcite crystal due to polymer complexation was significant and was followed on an Ångström scale without perturbing or removing the adsorbed molecules. The conformation of the 1,4- α linkage of the polysaccharide was found to alter the adsorbed amount and structure of the molecule at the interface. Alginate molecules containing high proportions of guluronate sugar residues adsorbed more and removed more calcium ions from the calcite surface when compared to alginate with a lower proportion of guluronate. This work shows the

1 importance of polymer conformation and ion affinity on adsorption to dynamic dissolving calcite
2 interfaces.

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Author Contributions

KLB, INS, and SMC designed the study. All authors were involved in performing the experiments and in the interpretation of the experimental results. The manuscript was written through contributions of all authors who have approved the final version of the manuscript.

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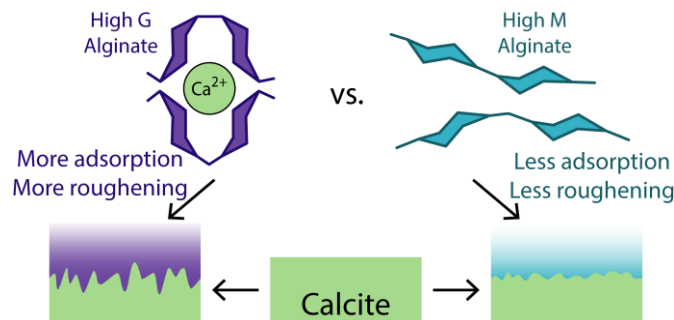
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7:Supplementary Material

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Highlights

- Sodium alginate adsorption to calcite in water was studied using neutron reflection
- Alginate formed highly hydrated layers at the surface with Gaussian distribution
- Alginates with high guluronic acid adsorbed more than with high mannuronic acid
- High guluronate alginates roughened the surface more due to complexation of calcium
- Added magnesium ions caused lower roughening and adsorption of alginate to calcite

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Credit Statement: The effect of alginate composition on adsorption to calcium carbonate surfaces

Kathryn Louise Browning Conceptualization, Methodology, Software, Investigation, Writing - Original Draft, Visualization

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